IMMUNOHISTOCHEMICAL MAPPING OF CCK IMMUNOREACTIVE CELLS IN SOME OF THE VITAL ORGANS OF *Clarias batrachus*, A FRESHWATER TELEOST

SYNOPSIS

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STATE OF THE ART

The gut is the first physiological state in all the living organisms with organ system grade of organization and it is responsible for enzymatic breakdown of complex dietary food into diffusible components which are then absorbed through intestinal mucosa. The whole digestive process is regulated by the harmonious function of two vital components, the enteric nervous system and the endocrine cells of the alimentary canal. The entire physiology of digestion is coordinated through mutual action of the enteric nervous system and the secretory functions of entero-endocrinocytes.

Gastrointestinal tract is the source of more than two dozen hormones. Among these most of the peptides are also found in the other tissues especially the brain. Cholecystokinin (CCK), is one such pioneer member of brain-gut peptide family discovered in the year 1928 in the extracts of jejunum tissues. It is basically known as a gall bladder contraction factor. CCK peptides are majorly produced and secreted by the enteroendocrine cells or I-cells located in the small intestine and cerebral neurons. Within the gut its distribution is unique because these are loacalized amidst the non-endocrine cell population, thus showing diffuse and dispersive nature of its distribution.

CCK is an anorexigenic peptide that has been identified through immunohistochemistry. They are now known to be present in different regions of the brain, gut, other ductless glands like pituitary, parafollicular cells of thyroid gland, Islets of Langerhans of pancreas and the adrenal gland, in the neurons of peripheral nervous system, in the renal cells, in the cardiomyocytes and in the different cells of the immune system. Moreover, the immune-reactive CCK receptors found localized in fish gonads (Nunzi *et al* 1985, Rehfeld *et al* 1976).

The available literatures suggest the pleiotropic roles of the hormone, its brain-gut and gonadal relationship, thus regulating the appetite and the reproductive physiology of the species.

However, much of the work regarding CCK has been done on mammals and a little attention has been paid to Indian teleosts. Due to above reasons and to fill the lacuna, the present investigation is designed to identify the CCK immunoreactive cells in the brain, gut and gonads of gastric omnivores fish *Clarias batrachus*.

International status:

The study of endocrine cells of the fish alimentary canal although has attracted the attention of a good number of workers for last 30 to 40 years only, but the present work is one of its kind in Indian context.

During the early years the immunocytochemical identification of gut endocrine cells has been studied by Langer (1979), Noaillac-Depeyre (1981), Holmgren (1982), Kazuyo Yoshida (1983), EL-Salhy (1984), Rombout (1986), Elbal (1987), Zhiping (1991), Kiliaan (1992), Pan (1993), Liqun (1994), Pan (1995), Pederzoli (1996), Reinecke (1997), Rajjo (1988), Bejenning (1988), Zhiping (1988), Pan (1988).

In recent years, CCK occurrence is reported in mammals by Konno (2015), Pyarokhi *et al* (2017) and Cardoso *et al* (2023). In case of Aves by Wan *et al* (2023). In amphibians by Rajjo (1988), and in fishes by Barrios *et al* (2020).

The immunohistochemical localization of CCK cells in gut and gut associated organs and extra gut regions such as brain, pituitary, thyroid, adrenal, kidney, and gonads in various vertebrates have been worked out by many researchers abroad. (Nunzi *et al* 1985, Rehfeld *et al* 1976.)

Vieira-Lopes *et al* (2013) have reported the presence of both the closed type and open type of CCK immunoreactive cells in the gastrointestinal tract of *Oligosarcus hepsetus* based on their immunohistochemical studies.

National Status:

Interestingly all the Indian researchers working on the gut physiology of teleost, have investigated only anatomy, histology and ultra-structural features of the gut. Mishra *et al* (1991), have worked on functional features of the gastrointestinal tract of *Lepidocephalicthys guntea* and Moitra *et al* (1988) upon its gut microanatomy and cytochemistry. Nagar and Khan (1957) have studied the anatomical and histological features of the alimentary canal of *Mastacembelus armatus* (Lacep).

The study of gut endocrine cells (Gastrin and Secretin secreting cells) through immunohistochemical method in Indian teleost and Indian Skipper frog was initiated by Shukla and Rao (2012 and 2015) and Rao and Shukla (2013).

PROBLEMS TO BE INVESTIGATED

The immunocytohemical study of CCK producing cell in the brain, alimentary canal and gonad of *Clarias batrachus* will help to address the following research queries:

1) Whether any relationship exists between the distribution and abundance of CCK IR cells with the feeding habits of *Clarias batrachus*?
2) What is the difference in the distribution of CCK IR cells during the feeding and fasting stages?
3) Is there any Brain-Gut cross talk through CCK in the digestive physiology of the fish?
4) Whether the distribution of the CCK IR only restricted to gut and brain tissues?
5) Whether there is any role of CCK in the fish reproduction and productivity?

OBJECTIVES OF INVESTIGATION AND MOTIVATION

The main objective is/are:

1) To identify the CCK producing cells in the brain, gonads and alimentary canal of *Clarias batrachus*, through immunohistochemistry.
2) To study the CCK immunoreactive cells during different phases of feeding and hunger.
3) To explore whether CCK influences reproductive physiology.

HYPOTHESIS

1. CCK is a key hormone that controls digestion and serves as an anorexigenic factor.
2. CCK is the satiety hormone regulating food intake and feeding behaviour of *Clarias batrachus*.
3. CCK also acts as novel crucial regulator of reproduction and establishes link between nutritional status and reproduction in fish.
4. CCK IR Cells are distributed throughout the different regions of gut, brain and gonads in *Clarias batrachus.*

RESEARCH DESIGN AND METHODOLOGY

The primary data of the distribution and relative abundance of CCK immunoreactivity will be generated through executing the IHC Protocol will be followed in the following manner experimental steps.

1) The animal model will be anesthetized by water soluble 2-Phenoxy ethanol.
2) The anesthetized animal will be dissected and subjected to trans cardinal perfusion to remove all the blood from the animal.
3) The injections of phosphate buffer saline and then after the suitable fixative will be given from the mouth of the fish into the lumen of the gut to allow it to pass out through the posterior opening of the tract to clear the gut content.
4) The desired portions of the alimentary canal, brain and the gonad will be cut into small pieces and immerged in the same fixative for overnight.
5) Two sets of tissues will be taken, for one set the block will be prepared in paraffin wax and for the other set block will be prepared in CRYO ZT compound.
6) Sectioning of the tissue will be done in normal microtome for paraffin block and in cryostat for frozen block.
7) Both the paraffin and cryo-sections will be subjected to Antigen Retrieval methods.
8) Upon the obtained sections the standard immunohistochemical methods will be carried out to get the result and will be compared with control sections.

Year-wise plan of work and targets to be achieved:

FIRST YEAR:

1) Sections of the brain, gonads and alimentary canal of different regions of *Clarias batrachus* will be prepared.
2) Standardization of the IHC protocol will be done.
3) Dilution test will be carried out, to determine the working dilution of primary antibody.
4) Standard Immunohistochemistry methods for the detection of CCK endocrine cells will be applied to identify CCK producing cells in the different segments of the alimentary canal and the gonads.
5) The number, size, shape, location etc., will be recorded during different phases of feeding.

SECOND YEAR: The above experiment will be repeated to confirm the result.

The tentative chapterization for the current study are as follows:

1. Introduction: Concept of Neuro-endocrine regulation of vertebrate gut physiology.
2. Historical Resume of Immunohistochemical localization of gut endocrine cells.
3. Materials and Methodology of IHC.
4. Results and Discussion
5. Conclusion.
6. Summary

TOOLS AND TECHNIQUES TO BE USED IN THE COLLECTION OF DATA

The experimental work of the proposed research will be done in the laboratory environment of Department of Zoology, St. Xavier's College, Ranchi, using instruments like cryostat and normal microtome to obtain the cryo-section as well as paraffin sections respectively.
Standardization of IHC protocol, Dilution test for determining the exact dilution of primary antibody for the experiment and Antigen Retrieval steps will be carried out to run the standardized protocol.
The designed protocol will require glassware and different chemicals mainly the primary polyclonal antibody against CCK, Secondary Conjugated Antibody kit EXTRA 3 kit, Chromogen: ACE 101 kit etc.

SIGNIFICANCE OF THE STUDY

The immunocytohemical study of CCK producing cell in the brain, alimentary canal and gonad of *Clarias batrachus* has the following significance:

1) This will help to understand the neuro-endocrinology of digestion and reproduction.
2) The findings will help to analyze the physiology of CCK action in gastric omnivore fishes.
3) This study will be useful in tracing the ontogeny of CCK producing cells in anaminotes, by comparing the results of published literature on the other species of anaminotes as well as aminotes.

4) Fish nutrition is important issue in fish farming as it is directly related with the fish production. Thus this investigation will help in formulating fish feed and increasing fish reproduction, productivity and propagation, required for each species of cultivable fishes.

RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED OF THE SOCIETY

Present day society is experiencing global food insecurity and therefore the sustainable development goal target 2 of 'Zero Hunger' is enshrined in the concept of Sustainable Development. The present study is linked with the fish feed and reproduction which is further related to fish productivity. If the productivity of edible fishes is increased, then global food insecurity can be resolved to some extent and one of the societal problem can be addressed.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

The aim of this research is to explore new facts which will enrich the existing knowledge. This present research focuses on the immunolocalization of CCK producing cells in some of the vital organs of the experimental fish model, which will help to know the pleiotropic roles of CCK hormone. This will assist in revealing the neuro-endocrine regulation of digestion and reproduction in a lower anaminote member and therefore contributing to enrich the existing knowledge in the field neuro-endocrinology, gut biology and reproductive physiology of fishes.

FUTURE WORK

Gastrointestinal tract is the source of more than two dozen hormones, some which are also found in the other tissues especially the brain as well as their receptors are also reported in gonadal tissues. In future work the immunohistochemical mapping of CCK receptors, Substance P, Peptide YY, NPY and Ghrelin like neuropeptides can be carried out to further explore the brain-gut-gonadal relationship for better understanding the regulation of digestive and reproductive physiology of fishes. The primary data generated by these researches will be useful in the management of fish reproduction and production to fulfil the protein rich dietary supplements for human consumption.

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SIGNATURE AND APPROVAL OF SUPERVISOR